

Amendments to the Specification:

Please amend the Title, beginning on page 1, line 5 and page 88, line 1, as follows:

GENES ENCODING NOVEL BACILLUS THURINGIENSIS PROTEINS WITH  
PESTICIDAL ACTIVITY AGAINST COLEOPTERANS

Please amend the Abstract, beginning on page 88, line 6, as follows:

The invention provides nucleic acids, and variants and fragments thereof, obtained from strains of *Bacillus thuringiensis* encoding  $\delta$ -endotoxins having pesticidal activity against pests of the order Coleoptera. The invention further provides mutagenized nucleic acids that have been modified to encode endotoxins having improved pesticidal activity and/or altered pest specificity. Particular embodiments of the invention provide isolated nucleic acids encoding pesticidal proteins, ~~pesticidal compositions~~, expression cassettes comprising such nucleic acids, and transformed ~~microorganisms and plants~~ and seeds comprising a nucleic acid of the invention. These compositions find use in methods for controlling pests, especially plant pests.

Please amend the paragraph beginning on page 14, line 14, as follows:

Some of the polypeptides of the invention, for example SEQ ID NOS:2 and 4 comprise full-length  $\delta$ -endotoxins; other polypeptides such as SEQ ID NOS:6, 8, 10, 16, 18, and 20 embody fragments of a full-length  $\delta$ -endotoxin; and SEQ ID NOS:12, 22, 24, 30, 32, 34, 40, 42, 44, and 46 provide polypeptide variants. Some of the polypeptide fragments and variants of the invention have enhanced pesticidal activity relative to the activity of the naturally occurring  $\delta$ -endotoxin from which they are derived, particularly in the absence of *in vitro* activation of the endotoxin with a protease prior to screening for activity. For example, the data presented herein in Table 1 of Example 6 indicates that the ~~NGRS~~ NGSR addition mutant (SEQ ID NO:12) of SEQ ID NO:16 (truncated 1218-1A endotoxin) is characterized by increased pesticidal activity against Colorado potato beetle.

Please amend the paragraph beginning on page 15, line 6, as follows:

SEQ ID NOS:12, 22, 24, 40, and 44 provide a family of polypeptides that embody variants of the 1218-1A truncated polypeptides set forth in SEQ ID NO:16, thus SEQ ID NOS:12, 22, 24, 40, and 44 provide variants (or mutants) of the biologically active fragment of the Cry8-like polypeptide set forth in SEQ ID NO:2. More specifically, SEQ ID NO:12 provides a mutant, referred to herein as NGSR.N1218-1, that comprises an additional trypsin-sensitive cleavage site; SEQ ID NO:22 provides a second mutant, referred to herein as LKMS.N1218-1, that comprises a chymotrypsin-sensitive cleavage site that is not present in the wild-type 1218-1 or 1218-1A polypeptide; and SEQ ID NO:24 provides a replacement mutant, referred to herein as LKMS.R1218-1, in which an existing trypsin cleavage-site is destroyed and a chymotrypsin site is introduced in its place. SEQ ID NO:40 provides a second chymotrypsin-addition mutant, referred to herein as LRMS.N1218-1, that comprises the alternative chymotrypsin cleavage site LRMS (SEQ ID NO:48). SEQ ID NO:44 provides a second replacement or substitution mutant, referred to herein as LRMS.R1218-1, in which the native trypsin site is replaced with the chymotrypsin cleavage site LRMS (SEQ ID NO: 48).

Please amend the paragraph beginning on page 15, line 21, as follows:

SEQ ID NOS:30, 32, 34, 42, and 46 provide a second family of polypeptides that embody variants or mutants of the truncated polypeptide set forth in SEQ ID NO:20. Thus, SEQ ID NOS: 30, 32, 34, 42, and 46 provide variants of the pesticidal fragment of SEQ ID NO: 2 that is set forth in SEQ ID NO: 20. More specifically, SEQ ID NO:30 provides a mutant, referred to herein as NGSR.N49PVD, that comprises an additional trypsin-sensitive cleavage site; SEQ ID NO: 32 provides a second mutant, referred to herein as LKMS.N49PVD, that comprises a chymotrypsin-sensitive cleavage site that is not present in the wild-type 1218-1 or 1218-1A polypeptide; and SEQ ID NO: 34 provides a replacement mutant, referred to herein as LKMS.R49PVD, in which an existing trypsin cleavage site is destroyed and a chymotrypsin site is introduced in its place. SEQ ID NO:42 provides a second chymotrypsin addition mutant, referred to herein as LRMS.N49PVD, that comprises the alternative chymotrypsin cleavage site LRMS (SEQ ID NO:48). SEQ ID NO:46 (LRMS.R49PVD) provides a second replacement or substitution

mutant in which the native trypsin site is replaced with the chymotrypsin cleavage site LRMS (SEQ ID NO: 48).

Please amend the paragraph beginning on page 20, line 12, as follows:

SEQ ID NO: 21 represents a *Cry8*-like nucleotide sequence that has been mutagenized to comprise 12 additional nucleotides (SEQ ID NO:25) that are not present in the wild-type endotoxin. The inserted nucleotide sequence was designed to encode an LKMS addition mutant that comprises a chymotrypsin cleavage site (LKMS) (SEQ ID NO:26) in the amino acid sequence of the encoded polypeptide. More specifically, the LKMS addition mutant (LKMS.N1218-1) comprises a nucleotide sequence insert that introduces the amino acid sequence LKMS (SEQ ID NO: 26) between amino acids 160 and 161 of SEQ ID NO:6. The LKMS replacement mutant LKMS.R1218-1 comprises a polypeptide in which the amino acid sequence LKMS (SEQ ID NO: 26) is introduced between amino acid 160 and 161 of SEQ ID NO:16 and the amino acids NGS are removed from amino acid positions 161-163 of SEQ ID NO:16. This modification removes a trypsin site and introduces a chymotrypsin site. Chymotrypsin cleaves bonds immediately C-terminal to Methionine.

Please amend the paragraph beginning on page 21, line 5, as follows:

It is recognized that any nucleotide sequence encoding the amino acid sequences NGSR (SEQ ID NO: 14), LKMS (SEQ ID NO: 26), or LRMS (SEQ ID NO: 48) can be used and that the exact identity of the codons used to introduce any of these cleavage sites into a variant polypeptide may vary depending on the use, i.e., expression in particular plant species. It is also recognized that any of the disclosed mutations can be introduced into any polynucleotide sequence of the invention that comprises the codons for amino acid residues that provide the native trypsin cleavage site that is targeted for modification. Accordingly, variants of either full-length endotoxins or fragments thereof can be modified to contain additional or alternative cleavage sites, and these embodiments are intended to be encompassed by the scope of the invention disclosed and claimed herein.

Please amend the paragraph beginning on page 26, line 16, as follows:

Briefly, the mutants provided herein include: mutants comprising a second trypsin cleavage site (i.e., NGSR (SEQ ID NO:14)) introduced into the amino acid sequence of the fragment presented in either SEQ ID NO:6 (1218-1) or SEQ ID NO:16 (1218-1A) or the fragment presented in SEQ ID NO:20 (49PVD). Mutants that comprise a chymotrypsin cleavage site comprising either the amino acid sequence LKMS (SEQ ID NO:26) or LRMS (SEQ ID NO:48) introduced in front of (e.g., directly 5' of) the trypsin cleavage site that is naturally present in the modified polypeptide sequence; and replacement mutants in which the native trypsin site that occurs in the toxin domain of the modified polypeptide is destroyed and a chymotrypsin site (e.g., LKMS (SEQ ID NO: 26) or LRMS (SEQ ID NO: 48)) is introduced in its place.

Please amend the paragraph beginning on page 27, line 6, as follows:

The NGSR mutants disclosed herein comprise an additional trypsin-sensitive protease site in a region of the amino acid sequence that encodes domain 1 of the polypeptide. For example, the NGSR.N1218-1 mutant (SEQ ID NO: 12) comprises an NGSR sequence (SEQ ID NO: 14) introduced between amino acid residues 164 and 165 of the wild-type protein. This amino acid sequence provides a second trypsin-sensitive cleavage site into the mutant endotoxin encoded by SEQ ID NO:11. More specifically, the NGSR (e.g., SEQ ID NO:14) sequence duplicates the endogenous trypsin cleavage site that is present at the target location, thereby introducing a second protease-sensitive sight into the loop region located between alpha helices 3 and 4 of domain 1. Thus, the amino acid sequence of SEQ ID NO:14, beginning at residue 160, ~~reads NGSRNGSR~~ comprises two contiguous copies of the sequence NGSR (SEQ ID NO: 14). In contrast, amino acid positions 160-164 of the wild-type protein comprise the sequence NGSR (SEQ ID NO:14), which is flanked by other sequences.

Please amend the paragraph beginning on page 69, line 2, as follows:

The NGSR samples, l-m, comprise a 1218-1 mutant polypeptide sequence that is set forth in SEQ ID NO:12 and designated "NGSR 1218-1." ~~NGSR 1218-1~~ This mutant sequence was

generated by the addition of an NGSR motif (SEQ ID NO: 14) to the amino acid sequence set forth in SEQ ID NO:16 after aa 164. More specifically, the NGSR mutant provides a 1218-1A mutant that includes the amino acid sequence NGSR (SEQ ID NO: 14) between aa 164 and aa 165 of the sequence set forth in SEQ ID NO:16. The addition of 4 residues to 1218-1A generated a protein with 673 aa. Bioassays of 1218-1A, 49PVD, and NGSR1218-1 indicated that all three protein samples are efficacious against Colorado potato beetle (CPB). Mutant NGSR1218-1 was found to be more potent than the parent 1218-1A and 49PVD mutant. The modified (e.g., truncated or mutant) 1218-1 polypeptides (49PVD, NGSR1218-1) were at least as active as the relevant 1218-1 or 1218-1A control sample.